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14. ABSTRACT

The highly pyrophilous "Little Ash Beetle" *Acanthocnemus nigricans* is attracted by forest fires and is equipped with one pair of unique prothoracic sensory organs. Our results have revealed that these organs most probably serve as infrared receptors. Each organ consists of a cuticular disc (diameter 150 µm) which is fixed over an air-filled cavity. On the outer surface of the disc, about 90 tiny cuticular sensory organs (sensilla) are situated. The poreless outer peg of each sensillum is 2 - 4 µm long and is surrounded by a cuticular wall. One ciliary sensory cell innervates the peg, the dendrite of which is divided into an outer and an inner dendritic segment. As a unique feature, the outer dendritic segment is almost totally replaced by an electron-dense rod, which most probably represents the hypertrophied dendritic sheath. The inner dendritic segment and the soma are fused indistinguishably forming a common cellular space. Thin, leaflike extensions of glial cells deeply extend into that enlarged lumen which also contains large numbers of mitochondria. The sensilla of the sensory disc of *A. nigricans* obviously represent a new type of insect sensillum. Electrophysiological investigations indicate that the sensilla function as warm receptors. Therefore, the prothoracic sensory organs of *Acanthocnemus* can be regarded as a micobolometer of reduced thermal mass. If the massive rod inside a disc-sensillum should function as a heat conducting structure, the *Acanthocnemus* IR organ can serve as a model for new uncooled IR sensor arrays having a size about one order of magnitude below the size of current microbolometer arrays.

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Final Performance Report

Introductory Remark:

The project focused on the infrared (IR) sensory systems of so-called pyrophilous beetles which show the behaviour to approach forest fires. For this reason, the beetles are equipped with IR sensory organs. Compared with uncooled technical IR sensors, the insect IR receptors have some interesting advantages: (i) there is no need for temperature constancy; (ii) the receptors can be operated at high ambient temperatures; (iii) the receptors are rugged having a smart design, and work even under harsh environmental conditions. Additionally, the neuronal network which processes the IR information in the central nervous system of the beetles, obviously can extract the relevant signals from considerable noise.

The objective of the proposed research was to broaden the understanding of the biological significance, special function, and performance of biological IR reception. The final aim of the research was to further develop concepts of uncooled IR sensors including the appropriate signal processing algorithms which are based on the principles and mechanisms of its biological models which were improved by millions of years of evolution.

To achieve the objectives, morphological as well as neurophysiological investigations have been performed. During the 12-month period of performance, work has focused on the newly discovered IR organs of the Australian "Little ash beetle", *Acanthocnemus nigricans* (Coleoptera, Acanthocnemidae).

People involved in this research:

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Eva Kreiss, PhD student

The prothoracic infared organs of pyrophilous beetle *Acanthocnemus nigricans* (Acanthocnemidae)

Acanthocnemus nigricans is a small dark beetle having a body length of 3-6 mm. The species *nigricans* is the only recent species within the genus *Acanthocnemus* and is distributed all over Australia. In contrast to its inconspicuous appearance, *A. nigricans* shows a remarkable behaviour as beetles of both sexes are attracted by forest fires. The beetles invade a freshly burnt area immediately after the flames are extinguished and approach areas where glowing remnants of trees or hot ashes are still present. The reason for this so-called pyrophilous behaviour is hardly understood but it can be speculated that even small "hot spots" serve as meeting places for the sexes. Most probably, the females deposit their eggs into the ash or under the bark of burnt trees. In feeding experiments with freshly hatched larvae, which have been made in February 2005 in Western Australia, we have discovered, that at least the first three instars are carnivorous.

Under the term of the grant, the ultrastructure of the "disc-sensillum" was investigated in great detail. After it turned out tat the about 90 sensilla which are situated on the outer surface of the prothoracic sensory disc represent a new type of insect sensillum (cf. Kreiss et al. 2005), we made an attempt to morphologically characterize the sensilla in order to understand their function. We focused on the morphology and ultrastructure of the dendritic region of the sensory cell because this part of a sensory cell is specially designed for stimulus uptake. In order to physiologically characterize the sensilla, we made electro-physiological recordings from single sensilla.

Material and methods

Animals

Adult beetles were collected in 2004 and 2005 on freshly burnt areas in Western Australia. Animals were kept for several weeks in plastic boxes and fed with raisins, peanuts and walnuts; drinking water was given ad libidum.

Light and transmission electron microscopy

Sensory discs of 12 beetles were isolated and immediately immersed in iced glutaraldehyde fixative (3% glutaraldehyde in 0.05 mol l^{-1} cacodylate buffer, pH 7.1;

osmolarity 380-400 mosmol l^{-1}) and fixed overnight. The discs were then washed in buffer, postfixed with 1.5% OsO_4 in the same buffer, dehydrated through an ascending ethanol series and embedded in Epon 812. Semithin and ultrathin sections were cut with a Reichert Ultracut Microtome using glass- or diamond knives. Semithin sections (0.5 µm) were stained with a 0.05% toluidine-blue/borax solution and examined with a Leitz DM RBE light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 109 TEM.

Electrophysiology

For electrophysiological recordings the legs of the beetles were removed and the insects were glued ventral side up on a platform with dental glue (pattern resin). Experiments were conducted at room temperature (22-23°C). Extracellular recordings from the cuticular sensilla were made with electrolytically sharpened tungsten electrodes (tip diameter < 1 µm). The electrode was inserted into the cuticle of the IR organ using a micromanipulator under visual control. A silver wire in the prothorax of the beetle served as reference. Neural activity was AC amplified 1000x (DAM 80, WPI), with filters set to 300 Hz (high pass) and 3000 Hz (low pass). Activity was displayed on an oscilloscope, digitised (CED Micro 1401 micro, Cambridge Electronic Design) and analysed on a PC using Spike 2 version 5.1 software (Cambridge Electronic Design), MS Excel and Origin 7.5 (OriginLab Corporation). The neural activity was always evaluated as instantaneous spike frequency. The standard deviation of the ongoing activity before stimulus onset was used as basis for our threshold criterion. A frequency higher than the ongoing activity plus three times the standard deviation was defined as a response to stimulation. Statistical tests were carried out using the programs "Salstat" (http://salstat.sunsite.dk/) and "statist" (http://wald.intevation.org/projects/statist/). All significance levels indicated represent two-tailed probabilities.

To test various stimulus modalities, we applied different kind of stimuli like acoustic or contact mechanical stimuli, a change in relative humidity or carbon dioxide. As acoustic stimuli we tested voice, hand clapping and computer-generated pure tone-sounds of different frequencies. Moving an eye lash over the surface of the disc and gentle application of an air flow served as mechanical stimuli. For thermal stimulation, we heated the disc organ of *A. nigricans* with a red helium-neon laser (λ= 632,8 nm).

The laser beam was adjusted via two mirrors onto one IR organ. Because the spot size was larger than the organ the receptors were always fully irradiated. The intensity was attenuated via neutral density filters from the maximal value (549 mW/cm^2) to zero. The laser power was measured with a Lab Master Ultima powermeter (Coherent). A camera shutter allowed defined exposure times.

Results

Morphology and ultrastructure of the sensilla

Series of cross and longitudinal sections through the sensilla revealed, that the small cuticular peg consists of massive cuticle. Neither pores nor a lumen inside the peg was found. From below, the peg is innervated by a single ciliary receptor cell (see reconstruction of a sensillum in Fig. 1). However, innervation is indirect. As a special feature, almost the entire DOS is replaced by a cylindrical rod of electron dense material which most probably is the hypertrophied dendritic sheath. The distal tip of the rod terminates at the base of the peg. At least in some sections, the dark material of the rod seems to invaginate somewhat into the basal part of the peg.

A ciliary constriction subdivides the tiny basal remains of the dendritic outer (DOS) and the dendritic inner segment (DIS). However, because the DOS shows a very small diameter (less than 1 µm), there is no decrease in diameter from the basal part of the DOS towards the dendritic constriction. Two basal bodies were found just below the constriction. The basal bodies were interconnected by root filaments which do not extend much further into the proximal region of the DIS. Below the ciliary constriction the DIS strongly broadens. As a predominant feature, many deep invaginations of the cell membrane into the lumen of the cell were observed. Thin, leaflike processes of glial cells are squeezed between the membranes and a large number of mitochondria were always found inside the DIS.

The soma region could be easily identified by a large nucleus but the DIS and the soma are not clearly distinguishable as they show the same structural features (i.e. thin but deep invaginations of glial cells into the cell lumen and lots of mitochondria). We found 2 - 3 enveloping cells (most probably representing the thecogen, trichogen and tormogen cell) which mainly enwrap the dendritic region and the distal region of the soma cell. Consequently, most of the cell soma is enveloped by a thin glial layer. In general, 2 - 3 nuclei of enveloping cells were found around the ciliary constriction.

Due to the extreme entanglement of cell material inside the disc, an unequivocal identification of single enveloping cells could no be made. Based on complete series of ultrathin sections through 5 sensilla, a graphical reconstruction of a single sensillum was made (Fig. 1).

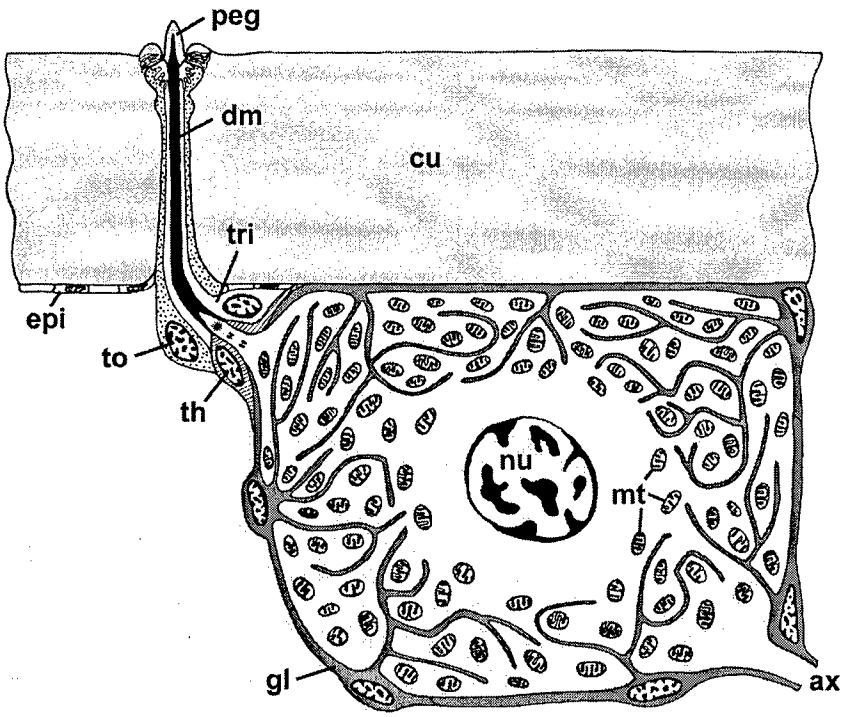


Fig. 1 Graphical schematic reconstruction of a sensillum of the sensory disc. The dendritic outer segment (DIS) is totally integrated into the apical region of the soma; two basal bodies indicated its distal ending. The short DOS is marked by an asterisk. Abbreviations: ax; axon; cu, cuticle; dm, electron dense material; epi, epidermis; gl, glial cell; mt, mitochondria; nu, nucleus; th, thecogen cell; to, tormogen cell; tri, trichogen cell.

To our knowledge, a sensillum containing a massive rod of electron dense material interconnecting the outer cuticular apparatus with an extremely reduced of the sensory cell has not been described in any other insect. Two interpretations are obvious: (i) the outer peg and the rod are without function. (ii) Because of its high density, the rod could serve as a heat conducting device conducting heat from the outer surface around the peg to the dendritic region of the sensory cell.

Electrophysiological recordings from the multipolar neurones inside the disc

The data reported here are based on extracellular recordings from 27 sensilla in 11 different beetles. Single sensilla spikes were usually observed after a shallow penetration of the cuticle; the resulting spike amplitudes lay between 0.5 and 1 mV. In some cases, the electrode additionally picked up small-amplitude spikes (<200 µV), originating probably from sensilla situated farther away from the recording electrode. Only large amplitude-spikes were evaluated (cf. Fig. 2). All sensilla recorded from the disc showed an ongoing activity with spike frequencies between 10 and 20 Hz at room temperature. 4 sensilla, however, exhibited a burst-like ongoing activity. Bursting sensilla did usually not respond to any stimulus presented indicating that they were probably injured by the electrode. These recordings were discarded and only recordings with stable firing rates and stable spike amplitudes were considered for analyses. Thermal stimuli, like warm air, IR irradiation from an IR-emitter and red laser illumination of the absorbing disc area, were effective to alter the firing activity of the sensilla. A typical response to laser illumination is shown in Fig. 2 (upper trace). The onset of the laser pulse was followed by a phasic-tonic increase in the spiking frequency, which persisted throughout the duration of the stimulus. This excitation was followed by a pronounced inhibition after switching off the laser. The inhibition was effective for 300 milliseconds, after which the cell returned to its resting spike frequency. None of the control stimuli tested had an effect on the firing rate of the sensilla (see "Material and Methods", and Fig. 2, lower trace).

The responses of 6 sensilla from 6 different beetles were quantified by plotting the instantaneous spike frequencies against time. The time interval chosen consisted of a 800 ms pre-stimulus, the 250 ms stimulus and a 550 ms post-stimulus interval for 22 irradiation intensities between 1.2 and 549 mW/cm^2. Fig. 3 shows a plot for one sensillum, which displayed an ongoing spike frequency of 12 spikes s^{-1}. The phasic response peaked around 50 spikes s^{-1} at the highest IR intensity of 549 mW/cm^2. The threshold for excitation was at 25 mW/cm^2 for this sensillum; this threshold also represents the lowest threshold found amongst the six sensilla investigated. The duration of the post-stimulus inhibition ranged between 80 and 320 ms varying with stimulus intensity.

To generalise, data were pooled by normalising the spike frequencies for each unit separately. The base for normalisation was obtained from mean spike frequencies in the 800 ms-interval before the stimulus onsets. Mean spike frequencies were then set to 100%. Pooled data are shown in Figure 3B; 9 instead of the 22 stimulus intensities are plotted for the sake of clarity. Phasic response portions reached up to 375 % of the resting activity level at the highest irradiaton intensity. Excitation maxima had a latency between 80 and 110 ms, inhibitory responses lasted between 110 and 310 ms. The average threshold for excitation was around 25 mW/cm^{-2}.

Plotting the peak frequencies against stimulus intensities for all six sensilla reveals linear response-intensity curves (Fig. 4, $0.93 \leq r \leq 0.98$, $p < 0.001$, Spearman rank correlation, $22 \leq N \leq 24$). The intensity-dependency of the six sensilla differs in both, the resting spike frequencies and the slope of the curves. For instance, sensillum 2 was spontaneously active at 9 spikes s^{-1} and reached a maximum phasic firing frequency of 30 spikes s^{-1}, resulting in a slope of 0,04 (spikes * cm^2) / (mW * s). Sensillum 1 starts off with a higher spontaneous firing frequency and reaches 74 spikes s^{-1}, yielding a much higher slope of 0,1 (spikes * cm^2) / (mW * s). The intensity curves did not saturate even at IR irradiation levels of almost 550 mW/cm^2; thus, the dynamic range of the investigated sensilla covers at least one order of magnitude of irradiation intensity.

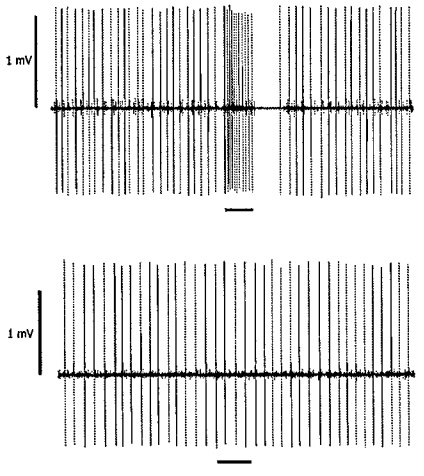
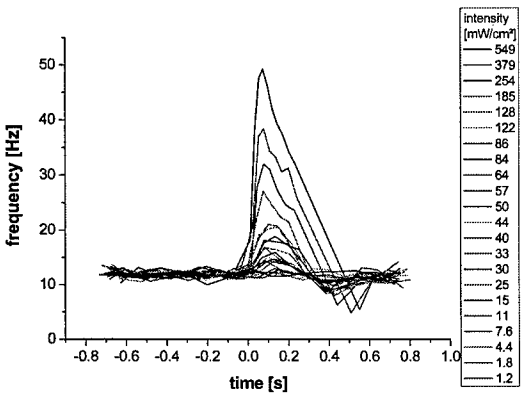


Fig 2: Upper trace: Example of an original recording. Response of a sensillum (large amplitude unit) to irradiation at 185 mW/cm^2. Duration of the stimulus (250 ms) is indicated by the black bar.
Lower trace: Activity of a sensillum (large amplitude unit) during a control experiment: Opening and closing the shutter alone did not alter the firing activity; thus, the sensillum did not respond to mechanical stimulation. Duration of the control stimulus (250 ms) is indicated by the black bar.

A



B

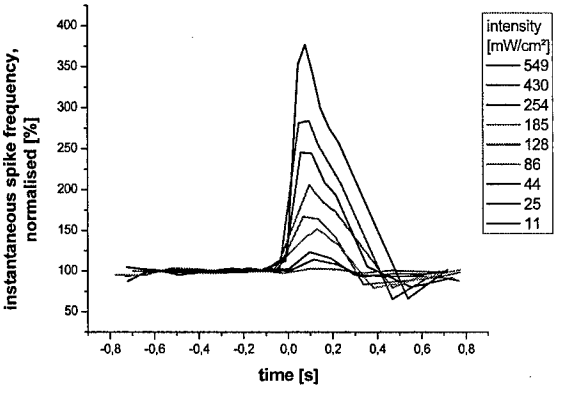


Fig 2: A and **B**) Averaged instantaneous spiking frequencies over time at 22 and 9 different intensities, respectively (t=0, onset of heating stimulus). The phasic response portions increase strongly with increasing intensities. Note the distinct inhibitory response component after the end of the stimuli.

A) Instantaneous frequencies of one single unit (N=6 sweeps per curve).

B) Normalised instantaneous frequencies of 6 units over time at 9 different intensities. The ongoing activity before stimulus onset was set to 100 % for each recording.

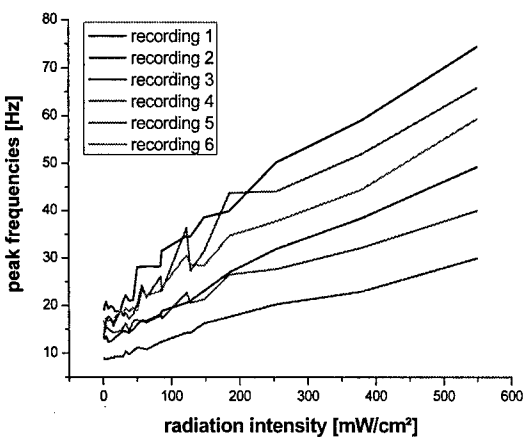


Fig 3: Peak frequencies of 6 units plotted against different stimulus intensities .The curves indicate a linear relationship between peak frequency and radiation intensity.

SUMMARY: *A. nigricans* has one pair of unique prothoracic sensory organs which most probably serve as infrared receptors. Each organ consists of a cuticular disc which is fixed over an air-filled cavity. On the outer surface of the disc, about 90 tiny cuticular sensilla are situated. The poreless outer peg of a sensillum is 2 - 4 µm long

and is surrounded by a cuticular wall. One ciliary sensory cell innervates the peg, the dendrite of which is divided into an outer and an inner dendritic segment. As a special feature, the outer dendritic segment is almost totally replaced by an electron-dense rod, which most probably represents the hypertrophied dendritic sheath. The inner dendritic segment and the soma are fused indistinguishably forming a common cellular space. Thin, leaflike extensions of glial cells deeply extend into that enlarged lumen which also contains large numbers of mitochondria. The sensilla of the sensory disc of *A. nigricans* obviously represent a new type of insect sensillum. Electrophysiological investigations indicate that the sensilla function as warm receptors. Therefore, the prothoracic sensory organ of *Acanthocnemus* can be regarded as a micobolometer of reduced thermal mass.

Comparison of the *Acanthocnemus* IR-organ with technical microbolometers: What can be learnt for the design of new IR sensors

In contrast to micromachined technical microbolometers where every single sensor (i. e. pixel) has an extremely reduced mass and, therefore, is thermally isolated from the substratum and from the neighbouring sensors, the 90 sensors (sensilla) on the outer surface of the disc are situated on a common auxiliary structure of low thermal mass (i.e. the disc). Considering this difference, the question arises what we eventually can learn for the design of new IR sensors. As already mentioned above, two possibilities are currently existing how the sensilla may work: (i) if the outer peg and the electron dense rod are without function, the temperature inside the disc is measured by the soma of the sensory cell and all receptors measure more or less the same temperature. In this case, the existence of many sensilla can be explained with an increase in sensitivity enabled by duplication of identical sensors. (ii) If the outer peg and especially the rod serve as a kind of heat conducting device, the heat energy taken up by the peg and its immediate surrounding is conducted to the thermoreceptive sensory cell. In this case, also a thermal mosaic of the outer disc surface can be measured. An inhomogeneous temperature distribution of the disc surface could originate form different alignments of the beetle's prothorax relative to the IR source.

One possible advantage of such a construction can be seen in a considerable reduction of sensor size which can be demonstrated by a simple calculation: the size

of a single pixel in current microbolometeres is 25 x 25 µm at best. For 90 pixels, this results in a total size of the sensor array of about 56,200 µm² (gaps between pixels not taken into account). The 90 sensilla on the *Acanthocemus* disc only need an area of about 7,700 µm² (diameter of the disc 140 µm, only half of the surface is covered by sensilla). Because the distance between sensilla is relatively large, it seems possible to further reduce the active sensor surface. Therefore, a reduction in sensor size in the dimension of one order of magnitude seems possible. If the electron dense rods really function as heat conductors (which has to be demonstrated), the design of a sensory array with a pixel size of only a few µm² seems possible.

Publications:

Kreiss E, Schmitz A, Schmitz H (2005): Morphology of the prothoracic disc and associated sensilla of *Acanthocnemus nigricans* (Coleoptera, Acanthocnemidae). *Arthropod Structure & Development* **34**: 419-428

Kreiss E, Gebhardt M, Schmitz H: Electrophysiological characterization of the infrared organ of the Australian "little ash" beetle *Acanthocnemus nigricans* (Coleoptera, Acanthocnemidae). [in prep.]

Kreiss E, Schmitz H (2005): The specialized sensilla of the Australian "little ash beetle" *Acanthocnemus nigricans*: morphological and electrophysiological characterisation. Arthropodenseminar "BUGS 2005", Bielefeld

Kreiss E, Schmitz H (2005): Responses of the prothoracic sensory organ of the Australian "Little Ash Beetle" *Acanthocnemus nigricans* to thermal and other kind of stimuli, 98. Ann. Meeting of the German Zoological Society (DZG), Bayreuth

Kreiss E, Schmitz A, Schmitz H (2004): The infrared organ of the Australian "little ash beetle" *Acanthocnemus nigricans*: morphology of the specialised sensilla. XXII International Congress of Entomology, Brisbane, Australien